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Third Molars as Risk Factors for Orofacial Pain. Findings from The SHIP Study

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"To be yourself in a world that is constantly trying to make you something else is the greatest accomplishment."

Ralph Waldo Emerson

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1 Introduction

Pain is a troublesome sensation associated with actual or potential damage and acts as a defense mechanism against potential and actual stimuli, which is crucial to our survival [1]. Pain was initially recognized as only a symptom, only for this belief to change later when it was realized that in a vast number of affected people there are no structural abnormalities or morphological changes which might cause this pain [2]. Certain features of pain have long puzzled clinicians and researchers, but the remarkable progress of pain research in the last decade has provided us with valuable insights into pain etiologies, signal transmission and underlying mechanisms [3,4]. Nonetheless, pain remains the primary reason for patients seeking health care [5].

1.1 Classification of pain

In order to establish a framework for standardized diagnostic procedures and potential therapeutic approaches, pain may be categorized by etiology (e.g. cancer pain), perceived location (e.g. orofacial pain) or affected anatomical system (e.g. neuropathic pain). Severity and duration of pain are decisive features that distinguish acute and chronic pain. Acute pain is usually characterized by sudden onset with short duration, whilst chronic pain is defined as pain that persists beyond the reasonable healing time for a specific injury [6].

A Task Force initiated by the International Association for the Study of Pain (IASP) and the World Health Organization (WHO) revised the current definition of chronic pain to complement the current version of the International Classification of Diseases (ICD 11) [7]. This new classification defined chronic pain as “pains that occur on at least 50% of the days during at least 3 months” replacing the former definition set by the IASP of six-month duration. This classification includes 7 groups, (1) chronic primary pain, (2) chronic cancer pain, (3) chronic posttraumatic and postsurgical pain, (4) chronic neuropathic pain, (5) chronic headache and orofacial pain, (6) chronic visceral pain and (7) chronic musculoskeletal pain.

This pragmatic and clinically applicable classification is based on the “multiple parenting” principle, which allows the same diagnosis to be listed under one category as “primary parent” but will be “cross-referenced” to other categories as “secondary parents”. For

example, temporomandibular disorders pain could be considered a primary type of pain, but is also a musculoskeletal pain and clearly one of the subsets of orofacial pain.

1.2 Orofacial pain

The American Academy of Orofacial Pain (AAOP) defines orofacial pain as pain around the eyes, above the neck and anterior to the ears as well as pain within the mouth [8]. Orofacial pain is considered an umbrella term that includes various subsets such as: masticatory musculoskeletal pain, pain related to temporomandibular joint disorders, intra-oral and dental diseases.

It has been reported that around 3.5 billion patients have untreated intraoral and dental diseases [9]. A predicted biological consequence of untreated dental diseases is pain. Epidemiologic data have reported that up to 45% of the population suffer of orofacial pain and up to 27.5% of its intraoral subset [9-11]. Compared to other pain categories, studies looking into intraoral and dental causes of orofacial pain have been less rigorous, with the exemption of pain related to temporomandibular disorders, leaving us with little or no data on other etiologies [12,13].

Due to the complexity of the region, diversity of underlying anatomical structures and unpredictable pain referral mechanisms, an accurate diagnosis and proper management of orofacial pain disorders may present a difficult challenge for the physician [14]. Some severe cases require a thorough examination, adequate imaging and may even call for a multidisciplinary approach involving a neurologist, an otolaryngologist and a dentist.

The role of dentists in treating orofacial pain remains often neglected by patients who tend to seek treatment from medical practitioners first [15]. Pain of dental origin may start as a short stabbing pain but can also progress to persistent dull pain based on its etiology. A key point in orofacial pain diagnosis is to inspect and rule out all possible underlying causes, which may refer the pain to other regions distant from the origin [16].

1.3 Third molars and associated pathologies:

Pain accompanying tooth eruption is familiar during the primary dentition stage. Such complaints are also quite common during the permanent dentition stage particularly around third molars as they are the last erupting and most frequently impacted teeth in humans

between the age of 17-21 years [17,18]. This condition is known as pericoronitis, which manifests as an inflammation, pain and swelling of the soft tissues surrounding the crown of a partially erupted tooth, including the gingiva and the dental follicle [19].

Tooth impaction is defined as an abnormal condition in which the tooth fails to erupt to its functional position. The incidence of impacted third molars varies enormously among populations from 10-70% with most studies suggesting that females have a higher incidence of impaction when compared to males [20,21]. The population-based study of health in Pomerania (SHIP) showed that 16.7% of the population in Northeastern Germany has at least one impacted third molar [22]. Possible reasons behind the impaction of third molars include lack of space, abnormal position and modern human diet [23].

It has been a common belief that third molars are linked to multiple pathologies in the oral cavity including caries, periodontal damage of the second molar, root resorption of adjacent tooth and cystic changes [24,25]. Some severe cases may even lead to compromised general health condition that requires hospitalization. Removal of impacted third molars is a common procedure in oral surgery. The decision to carry out this surgery should be based on a valid indication and must be the result of a comprehensive clarification of the patient including possible complications of the surgery. Despite the fact that third molars removal has been a standard procedure in dentistry for decades, there are no clear-cut on its spectrum and indications and the debate about the prophylactic removal of asymptomatic and pathology-free third molars still exist [26,27]. Advocates of prophylactic removal call for an early surgical removal to avoid such complications, whilst other physicians call this procedure a “public hazard” and claimed that up to 60% of the patients had their third molars removed for no valid reason [28].

This topic has been extensively discussed in the literature and summed up as well-established guidelines and indications for the removal of symptomatic third molars. However, these guidelines have a grey zone when it comes to association of pain and third molars in the absence of typical pathological symptoms and call for individual risk-benefit assessment. This has led to conflicting opinions among experts of the same discipline when presented with the same cases [29].

With little evidence-based reports, this scientific debate did not reach its goal of establishing consensus guidelines. For example, the German national guidelines for surgical removal of third molars updated in August 2019 “recommend” removing third molars in patients suffering atypical orofacial pain when an association can be found. Furthermore, a Cochrane

review published in 2016 found no evidence to support removal of asymptomatic third molars and called for further studies on a larger scale to prove this association [30].

1.4 Rationale and objectives

Patients with a pain complaint seek help at the dentist office on daily basis. The tremendous progress in understanding pain mechanisms from molecular-level findings to chair-side experiences has helped a vast number of patients overcome their pain. Nonetheless, the association of third molars and orofacial pain remains vague and questionable with no supporting representative studies.

This epidemiological study sheds the light on an important topic that has been long speculated but not truly examined. Our aim is to define the association of third molars with orofacial pain in a representative sample from Northeastern Germany. Furthermore, results of this study may be a valuable contribution to the current national German guidelines for surgical removal of third molars.

2 Materials and Methods

2.1 Study sample

We analyzed whole body magnetic resonance images (MRI) from the study of health in Pomerania (SHIP) [31]. 2333 participants took part in the 11-years follow-up examination (SHIP-2) and a new cohort (SHIP-Trend) included 4420 participants. All 6753 participants underwent a medical examination, an oral health examination, a health-related interview and a self-administered health- and risk factor-related questionnaire, whereby 2522 participants agreed to undergo an additional whole-body MRI examination [32]. Due to image artifacts in the head region, 40 images were excluded. Further exclusion criteria included participants taking NSAIDs (n = 212), opioids (n = 18) or analgesics (n = 108), as well as participants describing pain upon palpation of the lateral condyles, in dorso-cranial direction or upon palpation of the masticatory muscles (n = 336) resulting in a study sample of 1808 participants (Figure 1).

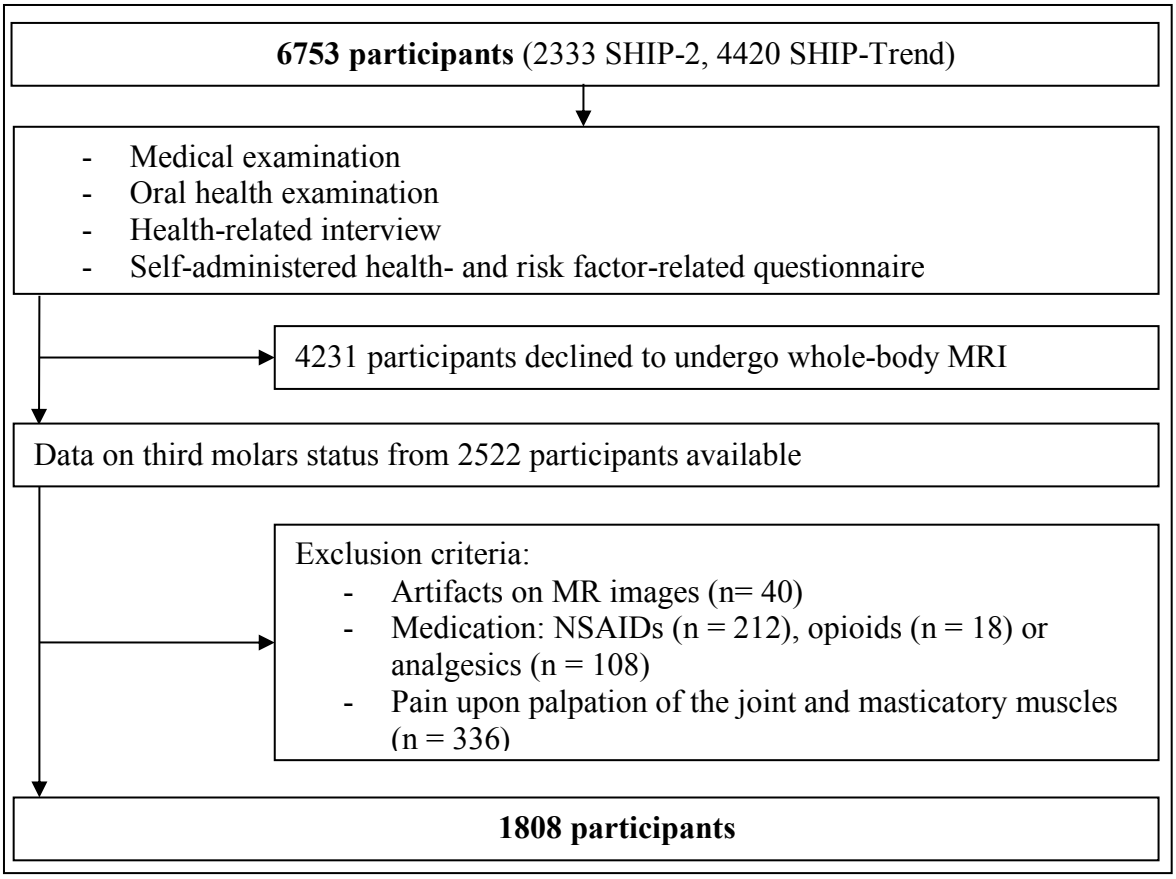

Figure 1. *Flowchart of study population*

2.2 MRI acquisition and third molars analysis

Magnetic resonance imaging scans were acquired using a 1.5T system (Magnetom Avanto; Siemens Medical Solutions). We used transversal T1-weighted turbo spin echo images (TE: 11 ms, TR: 587 ms, slice thickness: 4 mm, matrix: 256 × 256) and sagittal T1-weighted turbo spin echo images (TE: 120 ms, TR: 6760 ms, slice thickness: 4 mm, matrix: 448 × 448) to evaluate third molars status (Figure 2, 3).

MRI images were then examined by two trained dentists and third molars were classified according to Pell and Gregory as 1. missing, 2. erupted if its occlusal plane was above the cervical line of the adjacent second molar, 3. impacted if the occlusal plane was below the cervical line of the second molar. Inter-observer agreement was 98.5% for the impaction of third molars. Inter-observer agreement for third molars in the maxilla was a little higher (κ: .90-.94) than in the mandible (κ: .81-.83)

Figure 2. *T1-weighted MRI in the axial view*

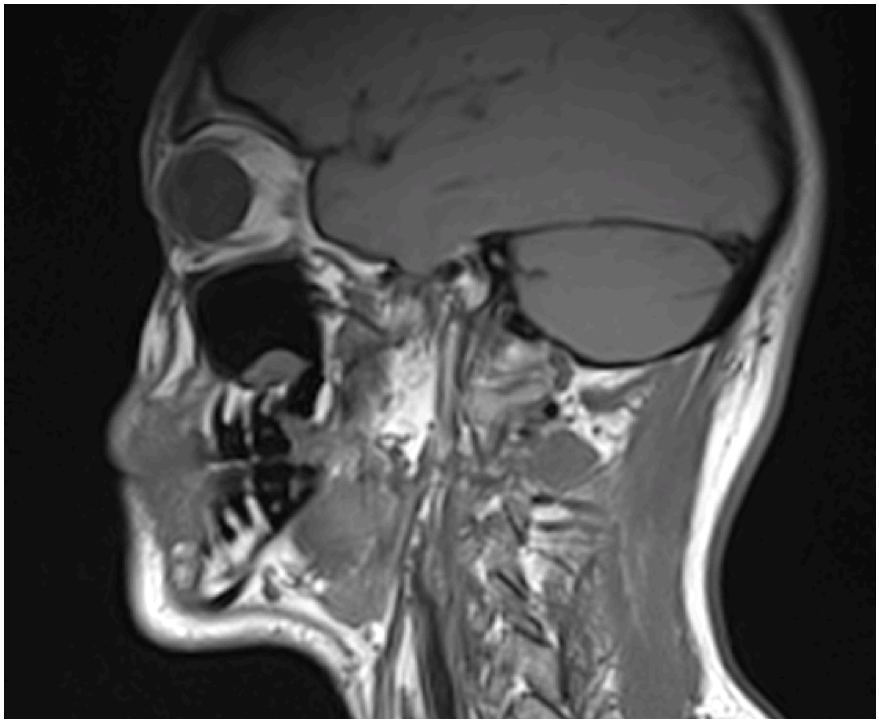

Figure 3. *T1-weighted MRI in the sagittal view*

2.3 Pain variables

Chronic orofacial pain was analyzed using a self-assessment questionnaire. Participants answered the question: '*Have you experienced any facial pain, masticatory muscle pain, pain in the temporomandibular joint or around the ears in the last 6 months?*' as well as migraine or other types of headaches.

Acute pain was inspected by palpating the masseter and temporalis muscles under pressure of about 1 kg/cm^2 bilaterally. TMD pain was defined as pain upon lateral and dorso-cranial palpation of the condyles.

2.4 Statistical analysis

Stratified by third molar status, categorical data were described as absolute numbers and percentages and continuous data as median, 25th and 75th percentile. Associations between third molar status and orofacial pain were analyzed by multivariable logistic regression models adjusted for age, gender, educational status and preferred chewing side.

'Erupted third molars' were used as the reference category for calculation of the odds ratios. In all analyses, a P value <.05 was considered as statistically significant. All analyses were carried out with Stata 15.1 (Stata Corporation).

3 Results

The incidence of impacted third molars was higher among younger participants. A total of 16% of all participants had at least one impacted third molar (n = 299), and 37.2% (n = 672) had no third molars at the time of examination. Higher impaction rates of third molars were observed in males than females for upper and lower jaw. In contrast, females had more missing third molars than males.

Impacted third molars in the maxilla are associated with orofacial pain (odds ratio 2.19; 95% confidence interval 1.19-4.02) (Figure 4, 5), whereas there was no such association for impacted third molars in the mandible (odds ratio 1.33; 95% confidence interval 0.74-2.37) (Figure 6). This association was more evident in the right upper jaw independent of age, gender, level of education and preferred chewing side. On the other hand, no significant associations of third molar status with migraine or other types of headaches were found. There were no significant interactions of age or gender with impacted or missing third molars on orofacial pain. We included caries and periodontal diseases as confounders in our analysis and found no differences.

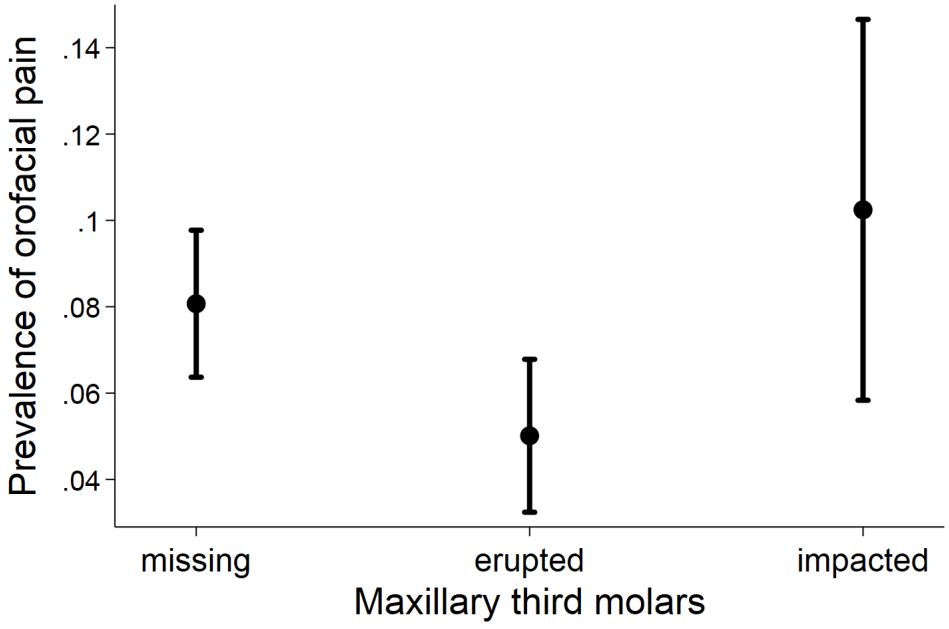

Figure 4. *Association of maxillary third molars with orofacial pain*

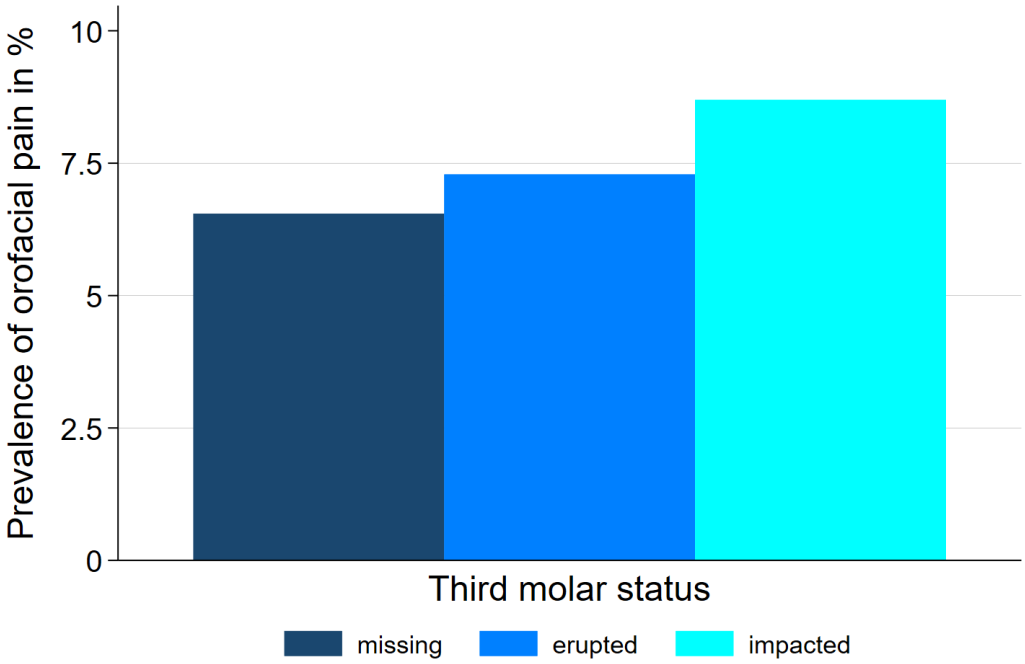

Figure 5. *Prevalence of orofacial pain based on third molar status*

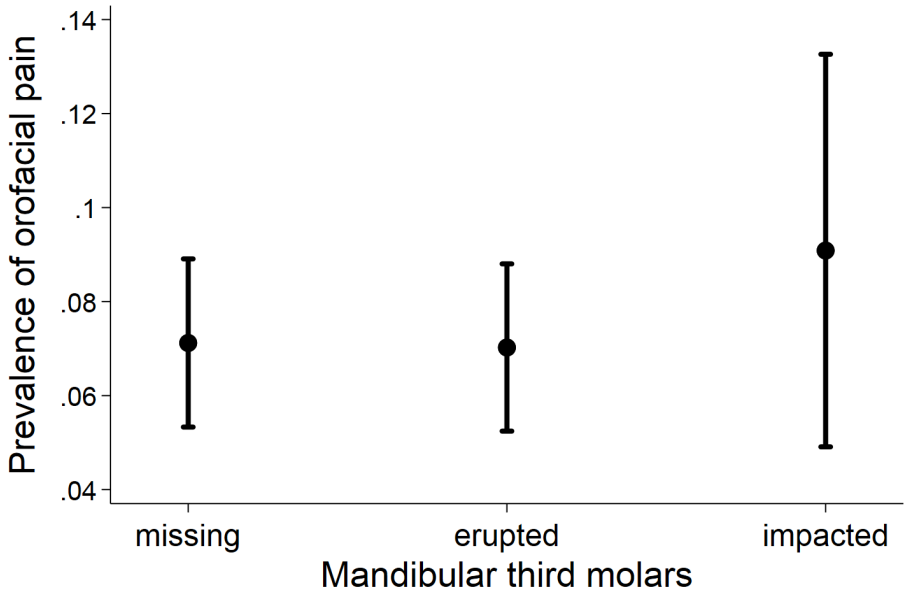

Figure 6. *Association of mandibular third molars with orofacial pain*

4 Discussion

Our results revealed a relatively strong association between impacted third molars and orofacial pain in a population-based sample. Furthermore, no association between third molars and migraine or other types of headaches was found. Despite the remarkable growth of knowledge and published research on orofacial pain and its etiologies, we found no studies that looked into this particular association.

Pain research has provided us with multiple classifications and standardized diagnostic procedures for a successful treatment through identification of biomarkers, pain mechanisms and risk factors. Woolf et al. published a prominent paper in 1998 where he categorized pain by its mechanism into: nociceptive, inflammatory, neuropathic and functional pain [33]. This categorization simplifies complex and multifactorial procedures that might overlap and cause pain. Etiologies of nociceptive and inflammatory pain are fundamentally different but both can offer plausible explanations of our results.

Nociception is our nervous system's response towards actual or potential harmful stimuli, which activates our sensory endings known as nociceptors [34]. The main responsible nociceptors are the Aδ and C-fibers. Aδ-fibers are the smallest myelinated nerves and have a relatively fast conduction velocity of 30 m/s and respond to thermal or mechanical stimuli. Such pain is perceived as sharp or stabbing pain similar to the one accompanying a partially erupting tooth. On the other hand, C-fibers are unmyelinated and have a relatively slow conduction velocity of approximately 2 μm/s and are stimulated by thermal, mechanical or chemical stimuli, which results in poor localization and dull pain sensation, a common feature among orofacial pain sufferers. Both fibers are mostly found in superficial organs such as the skin. However, C-fibers are additionally found in deeper organs such as the muscles and joints [35].

Over the last few decades, a variety of hypotheses have been put forward to explain the increasing rate of third molars agenesis and impaction in humans and some genetic loci were speculated to play a role [36,37]. We believe that impacted third molars might be perceived as potentially harmful stimuli, activating the nociceptors in the surrounding tissues and causing orofacial pain.

Inflammation is our tissues' response towards harmful stimuli [38]. This response induces the release of local chemical mediators, which in turn activate the nociceptors within the inflamed area [35]. Pericoronitis, periodontal damage and cystic changes might be considered as triggers

for inflammatory pain. The impact of gender on the incidence of pericoronitis has been largely discussed with conflicting results among populations [17]. Such variations were linked to treatment-seeking behavior and barriers to dental-care. On the other hand, inflammation of periodontal tissues in the third molars region can be difficult to eliminate properly and requires multiple therapy sessions [22]. A pervious study, of the same sample, published by our workgroup found no association between third molars and serum levels of inflammatory parameters [39]. Nevertheless, locally released chemical mediators might be just enough to trigger an action potential, subsequently causing inflammatory pain.

Our perceived results, in line with Woolf's approach, have a direct clinical implication in which the pain management strategy is aimed at eliminating the cause of pain, i.e. treatment of local inflammation induced by third molars or surgical removal of impacted third molars.

No association of mandibular third molars with orofacial pain was found, which might be due to morphological factors and anatomical differences between the maxilla and mandible such as bone type and vascularization [40]. Vascular supply of the bone has been intensively examined especially in the orthopedic field. High vascularity is linked to higher concentrations of nerve growth factor and local cytokines, which in turn have been reported to be essential to develop pain hypersensitivity [41]. We believe that the porous nature of the maxilla, especially in the tuberosity region, and its higher vascularity, compared to the mandible, facilitates the sensation and spread of pain, suggesting plausible justifications for our results.

Pain referral is common among pain patients and can be defined as the site of pain being different from the source of pain, which usually complicates the diagnosis procedure. The International Classification of Headache Disorders considered partially impacted third molars to be among the most common causes of orofacial pain, which may refer the pain to the head [42].

In light of the previous findings, it is important to remember that the perceived site of pain is a result of complicated underlying neurophysiological mechanisms such as activated peripheral receptors, neurotransmitters release and transmission and projection of nociceptive information into the central nervous system. In our case, pain caused by impacted third molars can be reported by the patient in other distant areas of the head and face.

It is not possible to identify which currently asymptomatic third molars will become symptomatic later. Unfortunately, the definition of symptomatic third molars is mostly limited

to local periodontal damage, root resorption, cysts and caries. A desired management approach is the watchful monitoring of third molars.

TMD pain and masticatory muscle pain are other critical factors that might overlap with orofacial pain and divert the diagnosis procedure [43]. Signs and symptoms of TMD can mimic those due to third molars in their perceived location. This might lead to unnecessary extractions of third molars when thought to be the cause of pain. Therefore, physicians are advised to perform a thorough and comprehensive oral examination including an assessment of the temporomandibular apparatus before setting their definitive diagnosis and therapy approach [44]. The diagnostic criteria for TMD (DC/TMD) provides a comprehensive assessment for TMD pain and should be considered as the golden standard while investigating the cause of orofacial pain. On the other hand, several studies reported an association of previous third molar removal and development of TMD signs and symptoms [45]. Plausible explanation is likely the long surgery duration requiring the patient to open their mouth widely for extended periods resulting in stretched muscles and ligaments, subluxation or disk displacement. Such complications are often overlooked in comparison to more immediate complications such as nerve injury and jaw fractures. This particular aspect must be accounted for when making risk-benefit-assessment for each individual patient suffering of orofacial pain.

On a final note, while some studies reported higher pain sensitivity among females with a female-to-male ratio of 4:1 for some types of headaches, we found no interactions of gender with impacted or missing third molars on orofacial pain or other types of headaches, conflicting other studies [46]. A possible aspect to consider is the differences in the perception of pain between genders, where some studies suggested higher self-reported pain among women [47] . Despite the pathophysiology behind this discrepancy still being unclear and complex, most studies linked higher pain perceptions with hormonal modulation of pain. Data on the association of pain with hormones' level and its magnitude are inconsistent and need further investigations.

The findings of this study set a starting point for future large-scale projects with more focus on third molars as possible causes of orofacial pain.

5 Conclusion

This study revealed a relatively strong association between impacted third molars and orofacial pain in a population-based sample, which has high clinical relevance. Epidemiologic data have reported that up to 45% of the population suffer from orofacial pain. People experiencing orofacial pain were 12 times more likely to report functional disability in their daily activities than their pain-free counterparts, thus posing a huge burden on the economical and health-insurance systems.

Patients suffering from orofacial pain are best managed in multidisciplinary centers where a team including dentists, neurologists and pain specialists can work together considering those results and employing them into developing novel treatment concepts. Decisions made by clinicians regarding removal of third molars should be evidence-based, rather than influenced by personal believes and biases.

The stimulation of pain from an impacted third molar, a normally non-painful stimulus, still needs further explanation to reach our ultimate goal of efficient and rationale management of the orofacial pain patient.

Our results suggest that impacted third molars, especially in the upper jaw, must be considered as a possible reason for orofacial pain and integrated into current national and international guidelines. Additional longitudinal studies are necessary to further confirm this association.

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ORIGINAL ARTICLE

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Are third molars associated with orofacial pain? Findings from the SHIP study

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Abstract

Objectives: To examine the association between third molars and orofacial pain. We hypothesized that impacted third molars are a cause of orofacial pain.

Methods: Magnetic resonance images of 1808 participants from two population-based cohorts from Northeastern Germany were analysed to define the status of third molars according to the Pell and Gregory classification. A self-reported questionnaire and a clinical dental examination were used to detect chronic and acute complaints of orofacial pain, masticatory muscle pain, migraine and other types of headache. Logistic regression models were used to analyse the associations between third molar status and orofacial pain.

Results: Individuals with impacted third molars in the maxilla had a higher chance of chronic orofacial pain than those with erupted third molars (odds ratio 2.19; 95% CI 1.19-4.02). No such association was detected for third molars in the lower jaw. Third molars were not associated with masticatory muscle pain, migraine or other types of headache.

Conclusions: Impacted maxillary third molars might be a cause of chronic orofacial pain. Thus, physicians should consider the eruption/impaction status of third molars in their decision-making process when treating patients who complain of orofacial pain.

KEYWORDS

epidemiologic studies, magnetic resonance imaging, orofacial pain, third molar, whole-body imaging

1 | INTRODUCTION

Pain is a crucial reaction against chemical, physical or harmful stimuli. Some severe cases require a thorough examination, adequate imaging and may even call for a multidisciplinary approach.[1] There are several ways to categorize pain, such as by cause (eg cancer pain), location (eg orofacial pain) or affected anatomical system (eg neuropathic pain). The intensity and nature of pain can be used to differentiate between acute and chronic pain. The International Association for the Study of Pain (IASP) and the World Health Organization (WHO) created a Task Force to complement the current version of the International Classification of Diseases (ICD 11) in respect of chronic pain. It defined chronic headache and orofacial pain as 'Pains that occur on at least 50% of the days during at least 3 months'.[2] Orofacial pain is defined as pain of the hard and soft tissues around the eyes or ears, as well as pain within the oral cavity.[3] It is a broad term that encompasses multiple subsets such as masticatory musculoskeletal pain, pain related to temporomandibular joint disorders, neuropathic pain, neurovascular pain, intra-oral and dental diseases,[4] which justifies the rapidly growing role of dentists in treating orofacial pain. Orofacial pain is relatively common, affecting up to 45% of the adult population, but barely half of those seek treatment.[5-7] It can arise from different tissues and aetiologies; masticatory muscle pain and temporomandibular joint disorders (TMD) are examples of pain originating from soft and hard tissues, respectively. Previously published studies focused on orofacial pain related to those conditions with considerable variation of reported numbers,[8,9] which were often justified by the different coping abilities and treatment seeking behaviour among populations.[10,11] Additionally, there are numerous dental diseases that can cause orofacial pain which can originate from the teeth, the surrounding periodontium, oral mucosa and other structures of the oral cavity.[12]

It has long been speculated that third molars contribute to headache disorders and orofacial pain.[13] They are the most frequently impacted teeth, and many are associated with cystic changes (up to 50% in some studies), periodontal damage (impacted third molars increased the risk more than 4-fold) and caries of the distal surface of second molars (up to 12%).[14-16] There are conflicting estimates of the frequency of neoplastic lesions associated with third molars,[17-19] thus casting doubts on claims of necessity to remove third molars to prevent pathological changes.[20,21] This has led to opposing opinions among oral surgeons and general dentists on the indications for third molar removal when presented with periapical radiographs of the same patient.[22] Although this topic has been extensively discussed in the literature and summarized as well-established guidelines,[23] the need for third molar removal is still debated. The International Classification of Headache Disorders viewed partially impacted third molars as among the most common causes of orofacial pain.[13] A 2016 Cochrane review which investigated the need for removal of asymptomatic impacted third molars found no evidence to support it and called for longer-term studies to clarify this matter.[24] The review defined third molars to be asymptomatic when signs of diseases affecting the tooth and nearby structures, such as root resorption and periodontitis, were absent, and there was no mention of a possible association with orofacial pain. Dogan et al[25] examined radiographs from 832 military recruits finding partially erupted third molars to be the most symptomatic. An investigation of orofacial pain and a history of third molar removal suggested that a history of third molar extraction is associated with orofacial pain.[26] There is little in the literature revealed on the association of impacted or erupted third molars with orofacial pain. Previous studies have restricted their assessment to local symptoms specifically caused by partially impacted third molars. Anatomical proximity means that pain caused by TMD can also mislead clinicians, and this was not accounted for in previous studies. Furthermore, recruiting patients from healthcare facilities compromises the generalizability of study findings.

Despite advances in understanding pain mechanisms and referral, the association between third molars and orofacial pain remains largely overlooked with knowledge based on limited data. Responding to these shortcomings, the present study aims to shed more light upon this association in a large representative sample of the population. We employed state-of-the-art MRI assessments to examine the status of third molars, and we assessed pain complaints through self-reported questionnaires augmented with oral clinical examinations to identify potential causes of orofacial pain. We hypothesized that individuals with impacted third molars have higher rates of orofacial pain.

2 | METHODS

We included participants from the Study of Health in Pomerania (SHIP), a cross-sectional population-based study assessing the prevalence and incidence of common population-relevant diseases and their risk factors in Northeastern Germany.[27] SHIP participants were randomly selected using public registries. Baseline examinations (SHIP-0) were conducted between 1997 and 2001, at which 4308 individuals participated. Follow-up examinations were undertaken at 5 years (SHIP-1; 2002-2006; 3300 follow-up participants, follow-up response 83.6%) and 11 years (SHIP-2; 2008-2012; 2333 follow-up participants, follow-up response 62.9%) after baseline.[27] In 2008, a new cohort (SHIP-Trend; 2008-2012) with 4420 participants was established in the same geographic region. Participants in both cohorts underwent a medical examination, an oral health examination, a health-related interview and a self-administered health- and risk factor-related questionnaire. Out of 6753 participants from both SHIP-2 and SHIP-trend, a total of 2522 participants (37.3%) agreed to undergo an additional whole-body MRI examination. Of those 2522 participants, 40 were excluded due to artefacts affecting the head region, resulting in MRI data on third molars from 2482 participants (98.4%). Excluded from this study were those taking NSAIDs (n = 212), opioids (n = 18) or analgesics (n = 108). Furthermore, to avoid confusion with signs of TMD, participants describing pain upon palpation of the lateral condyles, in dorso-cranial direction or upon palpation of the masticatory muscles were also excluded (n = 336) resulting in a study sample of 1808 participants (71.7%).

Magnetic resonance imaging scans used a 1.5T system (Magnetom Avanto; Siemens Medical Solutions). The complete whole-body MRI

protocol has been described previously.[28] For the evaluation of third molars, transversal T1-weighted turbo spin echo images (TE: 11 ms, TR: 587 ms, slice thickness: 4 mm, matrix: 256 × 256) and sagittal T1-weighted turbo spin echo images (TE: 120 ms, TR: 6760 ms, slice thickness: 4 mm, matrix: 448 × 448) were used. Additionally, coronal T2-weighted fat suppressed images (TR 4891 ms, TE 670 ms, inversion time 160 ms, slice thickness 5 mm) were available for further analysis of third molars. MR images were transferred to a working station (iMac 27″; Apple) where an open-source DICOM viewer (OsiriX v.3.8.1; Pixmeo) was used to load and analyse the MRI images (Figure S1).

Magnetic resonance imaging data were visually scrutinized by two trained dentists with a predefined Kappa algorithm used to measure inter-observer agreement; this was 98.5% for the impaction of third molars. Inter-observer agreement for third molars in the maxilla was a little higher (κ: .90-.94) than in the mandible (κ: .81-.83). Third molars were identified on the images and categorized according to Pell and Gregory[29] as missing, erupted or impacted. A third molar was considered erupted if its occlusal plane was above the cervical line of the adjacent second molar. Third molars with an occlusal plane below the cervical line of the second molar were considered impacted.[14]

Study and examination protocols were approved by the ethics committee at the University Medicine Greifswald (15.05.2008, BB 39/08). All participants signed an informed consent form, and investigations were undertaken in accordance with the Declaration of Helsinki.

Data on chronic orofacial pain were collected using a self-assessment questionnaire. Participants answered the question: 'Have you experienced any facial pain, masticatory muscle pain, pain in the temporomandibular joint or around the ears in the last 6 months?' They were further asked whether they had had migraine or other types of headache. Additionally, the masseter and temporalis muscles were palpated under pressure of about 1 kg/cm^2 bilaterally during an oral clinical examination, allowing an objective evaluation of acute masticatory muscle pain. We determined TMD pain using lateral and dorso-cranial palpation of the condyles. The lateral palpation of the TMJ was conducted with lateral pressure of about 2 kg/cm,^{2} while the mouth was slightly open. The dorso-cranial condyle compression occurred with the participant's mandible in the relaxed position. Participants were asked to describe their perception as 'painless', 'uncomfortable' or 'painful'. To distinguish pain and discomfort, each outcome (TMD pain and muscle pain) was defined as present if there was at least 1 site with pain upon palpation. The category 'uncomfortable' was not excluded but coded as 'painless'. This examination was part of the oral examination of the SHIP study and was performed by 8 trained, calibrated and certified dentists.[30] Examiners' training took place before the study started and twice a year during data acquisition. Inter-examiner variability for TMD signs was measured in 5 calibrated sessions using a total of 22 volunteers (7 of them with functional complaints). Kappa values for detecting tenderness upon palpation of the masticatory muscles and the temporomandibular joint ranged from 0.53 to 0.63. Training of the dentists and consensus discussions occurred before the start of the examinations and were repeated for calibration twice a year during the period of data collection.[31]

2.1 | Oral clinical examination

Coronal caries status was recorded as overt carious defects, fillings, secondary caries or missing teeth recorded at surface level (occlusal, mesial, distal, vestibular and oral) on a half-mouth basis excluding third molars. Coronal caries was identified visually using a periodontal probe according to the WHO criteria, but excluding third molars.[32] Using this information, the DFS scores were calculated. Periodontal examinations used a half-mouth approach on the left or right side (SHIP-2: alternatingly assigned; SHIP-Trend: randomly assigned), excluding third molars. Oral examinations used a half-mouth approach due to limited examination time per participant. Probing depth (PD) and clinical attachment loss (CAL) were measured with a periodontal probe (SHIP-2: PCP11, SHIP-Trend-0: PCP15; Hu-Friedy) at four sites per tooth (distobuccal, mesiobuccal, midbuccal and midpalatal/midlingual). Demographic data (ie gender and level of education), preferred chewing side and having a medical diagnosis of migraine, were reported through a computer-assisted interview.

2.2 | Statistical analysis

Stratified by third molar status, categorical data were described as absolute numbers and percentages and continuous data as median, 25th and 75th percentile. Associations between third molar status and orofacial pain were analysed using cross-tabulation logistic regression models, adjusted for age, gender, educational status and preferred chewing side. 'Erupted third molars' were used as the reference category for odds ratios. In all analyses, a P value <.05 was considered as statistically significant. All analyses were carried out with Stata 15.1 (Stata Corporation).

3 | RESULTS

Younger participants had more impacted third molars than their older peers (Table 1). A total of 16% of all participants had at least one impacted third molar (n = 299), and 37.2% (n = 672) had no third molars at the time of examination. Higher impaction rates of third molars were observed in males than females for upper and lower jaw. In contrast, females had more missing third molars than males.

We detected an association between impacted third molars in the maxilla and orofacial pain (odds ratio 2.19; 95% confidence interval 1.19-4.02) (Figure S2, Table 2), whereas there was no such association for impacted third molars in the mandible (odds ratio 1.33; 95% confidence interval 0.74-2.37) (Figure S3). This association was more evident in the right upper jaw and independent of the age, gender, level of education and preferred chewing side. On the other hand, no significant associations of third molar status with migraine or other types of headache were found. There were no significant interactions of age or gender with impacted or missing third molars

TABLE 1 Third molar status by sociodemographic and clinical characteristics

	N	Third molar status		
		Missing (n = 672)	Erupted (n = 836)	Impacted (n = 299)
Median age (25th, 75th percentile)	1808	61 (51; 69)	51 (42; 60)	47 (38; 61)
Sex	1808			
Male		326 (48.5%)	423 (50.5%)	187 (62.5%)
Female		346 (51.5%)	414 (49.5%)	112 (37.5%)
Education	1805			
Less than 10 y		187 (27.8%)	103 (12.3%)	35 (11.7%)
10 y		319 (47.5%)	509 (61.0%)	156 (52.2%)
More than 10 y		165 (24.6%)	223 (26.7%)	108 (36.1%)
Preferred chewing side	1802			
None		336 (50.0%)	420 (50.4%)	171 (57.8%)
Left		123 (18.3%)	125 (15.0%)	42 (14.2%)
Right		213 (31.7%)	289 (34.7%)	83 (28.0%)
Orofacial pain	1808			
No		628 (93.5%)	776 (92.7%)	273 (91.3%)
Yes		44 (6.6%)	61 (7.3%)	26 (8.7%)
Migraine	1825			
No		617 (91.8%)	749 (89.6%)	277 (92.6%)
Yes		55 (8.2%)	87 (10.4%)	22 (7.4%)
Headache	1568			
No		210 (37.1%)	248 (34.1%)	84 (32.7%)
Yes		356 (62.9%)	480 (65.9%)	173 (67.3%)
Periodontitis	1572			
None or mild		198 (39.8%)	340 (43.1%)	138 (48.4%)
Moderate		199 (40.0%)	297 (37.6%)	102 (35.8%)
Severe		100 (20.1%)	153 (19.4%)	45 (15.8%)
Dental caries	1721			
Mean DFS		17 (9; 25)	19 (12; 25)	15 (9; 24)

on orofacial pain. We included caries and periodontal diseases as confounders in our analysis and found no differences.

4 | DISCUSSION

We investigated third molar status in a population-based sample and found a relatively strong association between impacted maxillary third molars and orofacial pain. Interestingly, impacted third molars had no association with migraine or other types of headache. Although orofacial pain and its multifactorial causes and pathways have been extensively discussed in the literature, we found no previous studies that looked into this particular association.

Capitalizing on the large sample size and the representative character of this study, this is the first study to evaluate the association between third molars and orofacial pain using a combination of MRI diagnostics, questionnaires and clinical examinations. Our study sample was not recruited from a healthcare facility but rather randomly drawn from public registries, and the findings are likely to be generalized. Studies based on patients do not represent the situation in the general population. High levels of quality assurance and the strict adherence to standardization of the examination methods and data management are other advantages. Third molar status is most frequently analysed through two-dimensional X-ray images, whereby participants are exposed to radiation. Using MRI images, we were able to analyse third molars without additional radiation exposure. Additionally, we excluded participants suffering pain upon two palpation methods of the temporomandibular joint (TMJ) and masticatory muscles. This aimed to limit the possible overlapping with pain caused by TMJ disorders. Pain information was collected based on a combination of subjective and objective data, improving data validity significantly.

Unfortunately, by the time of data collection, it was not possible to know whether third molars were missing due to previous

extractions or congenital absence. Moreover, partially erupted and impacted third molars were rather difficult to distinguish and thus might have biased our findings. The reason for pain medication intake by our participants is unknown. This called for the exclusion of participants taking NSAIDs, opioids or analgesics.

Woolf et al[33] described four types of mechanism-based pain: nociceptive, inflammatory, neuropathic and functional pain. This classification simplified the complexity of different mechanisms behind pain sensation and suggests a number of interpretations of our findings. According to the International Association for the Study of Pain (IASP), 'nociceptive pain arises from actual or threatened damage to non-neural tissue and is due to the activation of nociceptors'.[34] Pain signals are transmitted through Aδ fibre and C-fibre. Aδ fibres are myelinated, fast-conducting and mostly found in superficial organs, whereas C-fibres are unmyelinated, slow-conducting and located in deeper organs such as the joints, muscles and bone.[35] Activation of Aδ fibres results in sharp well localized pain, such as that accompanying a partially erupted tooth. C-fibres, on the other hand, are responsible for prolonged dull painful sensations that are characterized usually by poor localization, a common feature among patients complaining of orofacial pain.

Third molar agenesis has been long studied, with some genetic loci speculated to play a role.[36] Many theories have been provided to explain the increasing rate of third molar agenesis and impaction through evolution, but this state is still controversial.[37,38] Regardless of the reason for impaction, an impacted third molar could be regarded as a potentially harmful stimulus and cause nociceptive pain. We believe that our observed association of orofacial pain with missing third molars can be explained by post-traumatic neuropathy secondary to the surgical removal of said teeth. Chronic pain following common surgical procedures is known in the medical field but remains neglected in dentistry.[39] Despite the fact that we were unable to differentiate between congenitally missing third molars and surgically removed ones, the reported low prevalence of third molars agenesis among various populations favours our proposed explanation.

Inflammatory pain, on the other hand, is caused by the chemical inflammation mediators produced locally by damaged tissues or released by inflammation cells migrating through the blood stream. Impacted third molars are often accompanied by cystic changes. Previous studies sponsored by the American Association of Oral and Maxillofacial surgeons proposed that even asymptomatic impacted third molars might pose high risks for adjacent second molars through the localized progression of periodontal disease and caries; this in turn may provoke a chronic inflammatory pain response.[40,41] However, in a previous study, we investigated the systemic effect of third molars on serum levels of inflammatory parameters and found no association.[42] Nevertheless, local inflammation due to either cystic changes around third molars or periodontal disease (even under its subclinical threshold) might amplify existing nociceptive pain.

Moreover, the cortical bone in the maxilla tends to be thinner than that of the mandible (The thickest cortical bone can be found in the mandible in the premolar and molar regions). This gives the maxilla its porous, flexible and highly vascular nature, in contrast to the dense compact mandibular bone.[43] Lower bone density has been reported in the tuberosity region, corresponding to the position of upper third molars. A higher vascularization rate reflects higher levels of nerve growth factor and the local cytokines that have been reported to be essential to the development of pain hypersensitivity.[44] Anatomically, the mandible represents the lower movable part of the face and articulates with the skull only through the temporomandibular joint, serving as the attachment point for various masticatory muscles. On the other hand, the maxillae form

TABLE 2 Association of third molar status with pain

	N (%)	Odds ratio (95% CI)	*P* > |z|
Orofacial pain	121 (7.7%)		
Molar 18			
Missing	87 (8.4%)	1.80 (1.10, 2.96)	0.020
Impacted	12 (11.3%)	2.41 (1.14, 5.09)	0.021
Molar 28			
Missing	89 (8.4%)	1.83 (1.10, 3.03)	.020
Impacted	11 (9.9%)	2.11 (0.98, 4.56)	.057
Molar 38			
Missing	67 (7.6%)	1.07 (0.71, 1.63)	.742
Impacted	14 (11.3%)	1.82 (0.94, 3.50)	.074
Molar 48			
Missing	68 (7.9%)	1.05 (0.70, 1.56)	.822
Impacted	7 (5.7%)	0.77 (0.33, 1.76)	.528
Maxilla			
Missing	74 (8.5%)	1.74 (1.10, 2.75)	.018
Impacted	18 (10.7%)	2.19 (1.16, 4.04)	.015
Mandible			
Missing	49 (7.5%)	0.99 (0.66, 1.49)	.975
Impacted	16 (8.7%)	1.25 (0.69, 2.27)	.460
Migraine	148 (9.4%)		
Maxilla			
Missing	85 (9.8%)	1.23 (0.83, 1.84)	.302
Impacted	15 (8.9%)	1.10 (0.58, 2.08)	.767
Mandible			
Missing	63 (9.6%)	1.00 (0.69, 1.45)	.986
Impacted	13 (7.0%)	0.79 (0.42, 1.51)	.479
Headache	912 (67.1%)		
Maxilla			
Missing	489 (65.3%)	1.09 (0.83, 1.44)	.524
Impacted	103 (72.0%)	1.34 (0.86, 2.10)	.200
Mandible			
Missing	378 (66.9%)	1.22 (0.94, 1.59)	.136
Impacted	116 (71.2%)	1.34 (0.89, 2.03)	.163

Note: Results are derived from logistic regression models adjusted for age, gender, educational status, preferred chewing side, periodontitis and caries with erupted third molars as reference category.

the dominant portion of the face and are connected with a number of skull bones. The difference between the maxilla and the mandible in our findings may be attributed to those anatomical characteristics. We detected a slight difference between the right and left side of the maxilla but this is unlikely to be important. We believe that complex associations among the previously mentioned mechanism-based pains, and the anatomical considerations of the upper and lower jaw are the underlying causes of orofacial pain from impacted maxillary third molars.

When examining the causes of orofacial pain, physicians should keep pain referral in mind through the diagnosis procedure. The complexity of the underlying anatomical structures and unpredictable pain referral mechanisms means that accurate diagnosis and proper management of orofacial pain disorders are a difficult challenge. A key point in orofacial pain diagnosis is to inspect and rule out all possible underlying causes which may have referred the pain to sites distant from the origin.[45] The actual source of orofacial pain might be distant from the location described by patients. The intensity, duration and nature of pain should help differentiate pain origin as part of the initial diagnosis. Pain caused by TMD is very likely to overlap and be confused with third molar complaints. DeAngelis et al[46] examined patients referred for third molar removal and suggested, on the contrary, that signs of TMD are common in patients referring for third molar removal. The guidelines of the American Academy of Orofacial Pain for assessment, diagnosis and management of orofacial pain and diagnostic criteria for temporomandibular disorders (DC/TMD) remain the gold standard for physicians differentiating both aetiologies.

The failure to diagnose and manage orofacial pain may lead to the development of chronic orofacial pain which in turn have a huge impact on patients' quality of life. Orofacial pain can prevent patients from performing their daily tasks and activities and led in some severe cases to depression.[47,48] Besides, the high percentage of patients not seeking professional treatment reported by many studies amount to unnecessary financial burden for healthcare providers and the economic system due to lost working days.[49,50] Patients are usually unsure where to start their treatment, which emphasizes again the importance of multidisciplinary work and the essential need of a dental consult to rule out causes of orofacial pain of dental origin in general and impacted third molars in particular.

We have highlighted a number of explanations for orofacial pain caused by third molars and thus will be valuable for practitioners in their decision-making on third molar removal. Dentists should be more involved in managing orofacial pain because they are well acquainted with the various underlying structures of the orofacial region, bearing in mind possible consequences of removing third molars or choosing to opt for active surveillance. Risk-benefit evaluation and possible complications associated with surgery should not be underestimated. An individual assessment for each patient with a pain complaint is essential for optimal care. Longitudinal studies focusing on orofacial pain and third molar occurrence are needed to confirm the associations reported here.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

MM, AD, OB, H-RM, HV and SK have made substantial contributions to conception and design of the study. MM, TI, PS, TK, RB and SK have been involved in data collection and data analysis. All authors have been involved in data interpretation, drafting the manuscript and revising it critically and have given final approval of the version to be published in this journal and agreed to be accountable for all aspects of the present work'.

COMPLIANCE WITH ETHICAL STANDARDS

Informed consent

Informed consent was obtained from all individual participants included in the study. This study was approved by the ethical committee of the University Medicine Greifswald (15.05.2008, BB 39/08). This research was conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Do Third Molars Contribute to Systemic Inflammation? Results From a Population-Based Study From Northeast Germany

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Purpose: Erupted and impacted third molars have been reported to contribute to systemic inflammation. This study investigated the systemic effect of third molars on serum levels of inflammatory parameters and on inflammatory messenger peptide hormones in a general population sample.

Materials and Methods: Data of 2,151 participants from the Study of Health in Pomerania were included in this study. Erupted or impacted third molars were assessed with whole-body magnetic resonance imaging at 1.5 T and associated with biomarkers of inflammation, lipid metabolism, glucose metabolism, and peptide hormones by linear regression. Models were adjusted for age, gender, smoking status, education, and type 2 diabetes mellitus.

Results: Neither erupted nor impacted third molars were associated with high-sensitivity C-reactive protein, white blood cell count, or fibrinogen as markers for systemic inflammation. Participants with erupted third molars had markedly lower serum levels of leptin (β coefficient, −2.47; 95% confidence interval [CI], −4.47 to −0.48), angiopoietin-2 (β coefficient, −135.1; 95% CI, 248.6 to −21.5), and ratio of angiopoietin-2 to tyrosine kinase with immunoglobulin-like loop epidermal growth factor homology domain 2 (β coefficient, −6.57; 95% CI, −13.06 to −00.7) than participants without third molars. No such associations were observed for impacted third molars.

Conclusion: The present results did not substantiate a relation between third molars and an increase in systemic inflammatory markers. Therefore, dental practitioners should be careful when considering this as the only indication for removal of third molars, especially in medically compromised patients. The results of this study showed that participants with erupted third molars had lower levels of messenger peptide hormones, such as leptin and angiopoetin-2.

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Erupted and impacted third molars have been reported to contribute to systemic inflammation.[1,2] High-sensitivity C-reactive protein (hsCRP), leukocyte counts, and fibrinogen are the most commonly used inflammatory markers in clinical practice.[3-5] Erupted third molars were positively associated with serum levels of CRP and interleukin-6 in 3 cross-sectional population-based studies.[1] Erupted third molars also have been described as risk factors for increased periodontal probing depths, which in turn, if found in the third molar region,[6-8] can induce localized inflammation.[1,8,9] This can lead to a systemic manifestation of inflammation.[10] Likewise, a higher risk for increased probing depths and for periodontal pathology has been described for asymptomatic impacted third molars.[8,11,12] Associations of chronic apical periodontitis with increased hsCRP levels have been reported.[13] Dentigerous cysts developed by liquid accumulation between the enamel epithelium and the crown of impacted third molars also promote local inflammation in the connective tissue.[14,15] This local inflammation is reported to lead to systemic inflammation and can even cause sepsis.[15]

Graziani et al[2] evaluated changes of systemic inflammatory markers after removal of impacted or semi-impacted third molars in a small case-and-control study with 40 patients. In that study, patients with impacted or semi-impacted third molars had higher levels of CRP and fibrinogen at baseline than the control group. Therefore, they concluded that removal of semi-impacted or impacted third molars would have beneficial effects on systemic inflammation, suggesting that, even without signs of a clinical infection, a low-grade systemic inflammation is clinically relevant. Moss et al[10] deduced that women of child-bearing age should be made aware of the systemic risks of oral inflammation caused by third molars, which was indicated by higher odds for preterm birth in women with third molars. Furthermore, increased CRP levels are associated with metabolic syndrome[16] and are reported to trigger the development of a proinflammatory state, leading to atherothrombosis.[16]

In systemic inflammatory disorders, messenger peptides, such as leptin, angiopoietin, and insulin-like growth factor 1 (IGF-1), seem to play a role.[17-19] Third molars have been locally associated with leptin, angiopoietin, and IGF-1, but a systemic manifestation was not investigated.[20-22] In a small study with 21 patients, the third molar group with inflamed pulps expressed the locally increased messenger peptide hormone, leptin, compared with controls without inflamed pulps.[20] Leptin is an important hormone secreted by adipose tissue and is described as a mediator of the inflammatory response.[17] Similarly, angiopoietin-1 and angiopoietin-2 (Ang-2) were expressed in tooth germs and ameloblastic tumors and seemed to participate in tooth development and odontogenic tumor progression by regulating angiogenesis.[21] In systemic inflammatory disorders, the importance of the angiopoietin and tyrosine kinase with immunoglobulin-like loop epidermal growth factor homology domain 2 (TIE-2) system has been described in many studies.[18] Not only does the messenger peptide IGF-1 regulate the metabolism of hard dental tissues through binding to the receptor of odontoblasts, cementoblasts, and cell colonies in the pulpal mesenchyme of third molars,[22] but the IGF system also seems to play a role in linking glucose metabolism with systemic inflammation.[19]

The systemic effect of third molars is clinically relevant for practitioners deciding whether to remove third molars, especially in medically compromised patients. This study aimed to clarify the impact of erupted and impacted third molars, diagnosed by magnetic resonance imaging (MRI), on systemic inflammation in a population-based sample.

Materials and Methods

The Study of Health in Pomerania (SHIP) is a population-based cohort study in West Pomerania in the northeastern region of Germany.[23] In a random cluster sample, participants 20 to 79 years old were analyzed.[24] At follow-up, 3,708 eligible participants were re-invited for a second examination (SHIP-2; 2008 to 2012[23]), in which 2,333 participated (follow-up response, 62.9%). From 2008 to 2012, a second independent cohort (SHIP-Trend[23]) was established, covering the same geographic recruitment region. Of a stratified random sample of 8,826 adults 20 to 79 years old, 4,420 participated in SHIP-Trend (response, 50.1%).[25] The local ethics committee at the University of Greifswald (Greifswald, Germany) approved the study protocols (15.05.2008, BB 39/08). The study was conducted in full accordance with ethical principles, including the World Medical Association's Declaration of Helsinki, and written informed consent was obtained from each participant.

The present study used pooled data of SHIP-2 (n = 2,333) and SHIP-Trend (n = 4,420).[23] In SHIP-2 and SHIP-Trend, MRI[26] examinations were conducted. Of the 6,753 participants of SHIP-2 and SHIP-Trend, data on third molars were available in 2,484. In 333 participants, data on at least 1 laboratory marker were missing, resulting in a study population of 2,151. Serum levels of angiopoietin, angiopoietin receptor TIE-2, IGF-1, IGF binding protein-3 (IGF-BP3), and leptin were available in only a subset of this population.

Education and smoking status were assessed by computer-assisted personal interviews. Education was subdivided into 3 categories (<10, 10, and >10 yr). Smoking was categorized as never, former, and current.

All MRI examinations were performed in a 1.5-T magnetic resonance scanner (Magnetom Avanto; Siemens Medical Systems, Erlangen, Germany). The imaging protocol was described elsewhere.[26] For evaluation of third molars, transversal T1-weighted turbo spin echo images (echo time [TE], 11 ms; repetition time [TR], 587 ms; slice thickness, 4 mm; matrix, 256 × 256) of the neck and sagittal T1-weighted turbo spin echo images (TE, 120 ms; TR, 6,760 ms; slice thickness, 4 mm; matrix, 448 × 448) of the cervical spine, which included the maxilla and mandible, were used. In addition, coronal oriented T2-weighted fat-suppressed images (TR, 4,891 ms; TE, 670 ms; inversion time, 160 ms; slice thickness, 5 mm) were available. For third molar analysis, MR images were transferred to a work station (Mac OSX), and OsiriX 3.8.1 software (Pixmeo, Geneva, Switzerland) was used for analysis. Third molar analysis was performed by 2 trained dentists (P.S. and S.H.). Interobserver agreement κ values for third molar assessments ranged from 0.90 to 0.94 for the maxilla and from 0.81 to 0.83 for the mandible. In cases with disagreement, the assessment of the dentist with more radiologic experience and more involvement in the development of the protocol (P.S.) was used.

Image analysis of third molars was conducted with a predefined algorithm. Axial and sagittal images were displayed simultaneously, and the cross-referencing tool of OsiriX was used for exact anatomic correlations. For adequate third molar analysis, sagittal images were zoomed. On axial images, the number of teeth was counted for each quadrant, and the molars were identified. The levels defined by Pell and Gregory[27] were used to classify existing third molars of the mandible and maxilla. A 3-category classification for third molars resulted: *1)* nonexistent third molar; *2)* erupted but not impacted third molar; and *3)* at least 1 impacted third molar. In addition, upper third molars were evaluated as localized in the maxillary sinus if at least 50% of an impacted third molar was located in the maxillary sinus.

Blood samples were taken after a fast from 7 AM to 2 PM and analyzed in the central laboratory of the University Medicine Greifswald. Fasting blood samples were drawn from the cubital vein in the supine position, and serum aliquots were prepared for immediate analysis and storage at −80°C. Ang-2 and soluble TIE-2 (sTIE-2) levels were measured in serum using commercially available assays (R&D Systems, Minneapolis, MN) using 3 different lots in the 2 study populations. The minimum detectable dose was 1.20 pg/mL for Ang-2 and 0.001 ng/mL for sTIE-2. Fibrinogen concentrations were determined in citrate plasma according to Clauss using a BCS-XP system (Siemens Healthcare Diagnostics, Eschborn, Germany).[28] White blood cell concentrations were measured within 60 minutes on a Sysmex XT-2000 analyzer (Siemens AG, Munich, Germany) in SHIP-Trend. HsCRP concentrations were measured using the Dimension VISTA 500 analytical system (Siemens AG). Photometry (Dimension VISTA; Siemens Healthcare Diagnostics) was used to measure total cholesterol, total triglyceride, and serum glucose concentrations. High-density lipoprotein (HDL) and low-density lipoprotein cholesterol were selectively precipitated and then determined by homogenous assays (Dimension VISTA). Serum leptin concentrations (Mediagnost, Reutlingen, Germany) were measured using the enzyme-linked immunosorbent assay technique. Glycated hemoglobin (HbA_{1c}) was determined by high-performance liquid chromatography (Bio-Rad, Munich, Germany).[29]

Stratified by third molar status, continuous data were described by medians and 25th and 75th percentiles and categorical data were described by absolute numbers and percentages. Linear regression models adjusted for age, gender, smoking status, and education and weighted for dropout from MRI examinations were used to associate third molar status with laboratory biomarker levels, with no third molars as the reference category. A P value less than .05 was defined as statistically significant. All analyses were conducted with STATA 14.1 (Stata Corporation, College Station, TX).

Results

Participants without third molars were older than those with erupted or impacted third molars (Table 1). Impacted third molars were more often observed in men than in women, whereas women more frequently had erupted third molars than men. Median serum levels of leptin and Ang-2 were lower in participants without third molars than in those with erupted or impacted third molars. Median levels of IGF-1 and IGF-BP3 were highest in participants with impacted third molars.

In multivariable linear regression adjusted for age, gender, smoking status, education, and type 2 diabetes mellitus, no relevant associations were observed between third molars and inflammatory or lipid markers. A sensitivity analysis stratified for periodontitis was performed, but no effect on hsCRP was discovered in the group with periodontitis or the group without periodontitis. Participants with third molars had notably lower levels of HbA_{1c}, leptin, Ang-2, and Ang-2/TIE ratio than those without third molars, whereas no such associations were observed for impacted third molars (Table 2). Associations of impacted third molars with IGF-1 and IGF-BP3 levels barely missed statistical relevance (Table 2).

Table 1. STUDY CHARACTERISTICS STRATIFIED BY THIRD MOLAR STATUS

	n	No Third Molars (n = 695)	Erupted Third Molars (n = 1,054)	≥1 Impacted Third Molar (n = 402)
Age (yr)	2,151	56 (45; 66)	50 (42; 59)	46 (38; 59)
Men	2,151	301 (58.7)	498 (47.3)	238 (59.2)
Current smokers	2,151	134 (19.3)	229 (21.8)	87 (21.6)
Type 2 diabetes	2,151	55 (8.0)	63 (6.1)	27 (6.8)
Education	2,117			
Low		146 (21.2)	121 (11.7)	47 (11.9)
Median		352 (51.2)	619 (59.9)	212 (53.7)
High		190 (27.6)	294 (28.4)	136 (34.4)
Waist circumference (cm)	2,151	89.3 (79.5; 99.0)	88.9 (80.0; 97.8)	89.0 (78.9; 98.0)
hsCRP (mg/L)	2,079	1.17 (0.64; 2.45)	1.13 (0.61; 2.39)	1.05 (0.57; 2.38)
Fibrinogen (Clauss; g/L)	2,151	3.0 (2.5; 3.5)	3.0 (2.4; 3.4)	2.8 (2.4; 3.4)
White blood cell count (Gpt/L)	2,151	5.8 (4.9; 6.8)	5.6 (4.8; 6.8)	5.6 (4.7; 6.7)
Glucose (mmol/L)	2,151	5.3 (4.9; 5.9)	5.3 (4.9; 5.8)	5.3 (4.9; 5.8)
HbA_{1c} (%)	2,151	5.3 (5.0; 5.7)	5.2 (4.9; 5.6)	5.2 (4.8; 5.5)
Total cholesterol (mmol/L)	2,151	5.4 (4.7; 6.1)	5.5 (4.8; 6.3)	5.3 (4.6; 6.1)
LDL cholesterol (mmol/L)	2,151	3.3 (2.8; 3.9)	3.4 (2.7; 4.0)	3.3 (2.7; 3.9)
HDL cholesterol (mmol/L)	2,151	1.4 (1.2; 1.7)	1.4 (1.2; 1.7)	1.4 (1.2; 1.6)
Triglycerides (mmol/L)	2,151	1.4 (1.0; 2.0)	1.4 (0.9; 2.0)	1.3 (0.9; 2.0)
Leptin (ng/mL)	759	12.4 (7.0; 23.5)	10.1 (5.7; 18.8)	7.6 (4.5; 13.4)
IGF-1 (ng/mL)	759	134 (103; 169)	142 (118; 174)	157 (120; 186)
IGF binding protein-3 (ng/mL)	759	4,097 (3,450; 4,763)	4,267 (3,731; 4,852)	4,343 (3,688; 5,106)
Angiopoietin-2 (pg/mL)	1,443	1,854 (1,424; 2,473)	1,722 (1,370; 2,248)	1,690 (1,246; 2,109)
Angiopoietin receptor TIE-2 (ng/mL)	1,443	18.0 (15.4; 21.1)	18.1 (15.6; 21.1)	18.5 (16.0; 21.9)
Angiopoietin-to-TIE ratio	1,443	106 (78; 141)	95 (73; 125)	89 (66; 120)
Periodontitis	2,151			
None or mild	959	287 (41.3)	473 (44.9)	199 (49.5)
Moderate or severe	1,192	408 (58.7)	581 (55.1)	203 (50.5)

Note: Data are expressed as median (25th percentile; 75th percentile) for continuous data or as absolute number (percentage) for categorical data.

Abbreviations: HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IGF, insulin-like growth factor; LDL, low-density lipoprotein; TIE-2, tyrosine kinase with immunoglobulin-like loop epidermal growth factor homology domain 2.

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Discussion

Neither erupted nor impacted third molars were associated with signs of a systemic inflammation in the general population. Associations of impacted third molars with the IGF system barely missed statistical relevance. Participants with erupted third molars had considerably lower serum levels of HbA_{1c}, leptin, Ang-2, and Ang-2/TIE ratio than those without third molars. No such associations were observed for impacted third molars.

In contrast to previous studies,[1,2,10] no relevant association between third molar status and inflammatory markers was found in the present study. Offenbacher et al[1] described an association of an increased probing depth in the distal sites of adjacent second molars with increased serum levels of interleukin-6 and CRP. The authors previously investigated the association between third molars and periodontal damage of adjacent second molars as a potential manifestation of local inflammation and found relevant associations in the mandible.[8] Calibrated and licensed dentists clinically measured probing depth and clinical attachment level with a periodontal probe. In the present analyses, no relevant association of third molar status with hsCRP levels was found in participants with periodontitis or in participants without periodontitis.

Methodologic differences could explain the discrepant findings between the present study and previous studies. In the study by Offenbacher et al,[1] impacted third molars were not evaluated by

Table 2. LINEAR REGRESSION RESULTS EVALUATING CROSS-SECTIONAL ASSOCIATIONS BETWEEN THIRD MOLAR STATUS AND SEVERAL BIOMARKERS

	Model 1	Model 2	
	≥1 Third Molar Available*	Erupted Third Molars*	≥1 Impacted Third Molar*
Waist circumference (cm)	0.65 (−0.52 to 1.82)	0.61 (−0.61 to 1.84)	0.74 (−1.00 to 2.48)
hsCRP (mg/L)	−0.17 (−0.77 to 0.43)	−0.21 (−0.86 to 0.44)	−0.06 (−0.69 to 0.56)
Fibrinogen (Clauss; g/L)	0.03 (−0.06 to 0.11)	0.01 (−0.08 to 0.10)	0.08 (−0.03 to 0.19)
White blood cell count (Gpt/L)	0.00 (−0.23 to 0.23)	−0.01 (−0.22 to 0.20)	0.03 (−0.38 to 0.44)
Glucose (mmol/L)	−0.03 (−0.24 to 0.18)	−0.07 (−0.26 to 0.12)	0.07 (−0.29 to 0.42)
HbA_{1c} (%)	−0.10 (−0.18 to −0.01)†	−0.10 (−0.18 to −0.02)†	−0.08 (−0.21 to 0.04)
Total cholesterol (mmol/L)	0.09 (−0.02 to 0.21)	0.12 (−0.01 to 0.24)	0.03 (−0.14 to 0.21)
LDL cholesterol (mmol/L)	0.06 (−0.03 to 0.16)	0.07 (−0.03 to 0.18)	0.04 (−0.11 to 0.19)
HDL cholesterol (mmol/L)	0.02 (−0.02 to 0.06)	0.02 (−0.01 to 0.06)	0.00 (−0.05 to 0.05)
Triglycerides (mmol/L)	−0.00 (−0.13 to 0.12)	−0.03 (−0.16 to 0.11)	0.05 (−0.15 to 0.25)
Leptin (ng/mL)	−2.47 (−4.47 to −0.48)†	−2.73 (−4.84 to −0.63)†	−1.72 (−4.09 to 0.65)
IGF-1 (ng/mL)	4.26 (−2.45 to 10.97)	2.80 (−4.12 to 9.73)	8.51 (−1.34 to 18.36)
IGF binding protein-3 (ng/mL)	100.8 (−47.2 to 248.7)	69.0 (−84.0 to 221.9)	193.6 (−15.7 to 403.0)
Angiopoietin-2 (pg/mL)	−130.1 (−241.6 to −18.5)†	−135.1 (−248.6 to −21.5)†	−115.5 (−283.2 to 52.2)
TIE-2 (ng/mL)	−0.15 (−0.71 to 0.40)	−0.20 (−0.78 to 0.37)	−0.00 (−0.77 to 0.76)
Angiopoietin-to-TIE ratio	−6.53 (−13.05 to −0.02)†	−6.57 (−13.06 to −0.07)†	−6.43 (−17.31 to 4.45)

Note: All regression models were adjusted for age, gender, smoking status, education, and diagnosed type 2 diabetes mellitus and weighted for dropout. Data are presented as β coefficient (95% confidence interval).

Abbreviations: HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IGF, insulin-like growth factor; LDL, low-density lipoprotein; TIE-2, tyrosine kinase with immunoglobulin-like loop epidermal growth factor homology domain 2.

* Compared with no third molar.

† $P < .05$.

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radiographic imaging or MRI. The present findings also differ from the comparative study of Graziani et al[2] in which participants with impacted or semi-impacted third molars had higher levels of CRP and fibrinogen at baseline than the control group. Therefore, Graziani et al concluded that third molar removal could have beneficial systemic effects. Selection bias could explain the discrepant results in the present study. Graziani et al[2] primarily investigated the influence of third molar removal on systemic inflammation in 18 patients with semi-impacted third molars and only 2 patients with impacted third molars who were referred to the Department of Dentistry and Oral Surgery of the University of Pisa (Pisa, Italy) for third molar removal. Conversely, in the present study, a possible misclassification between partially erupted and impacted third molars by MRI could have occurred.[8] However, no association with inflammatory parameters was discovered for impacted or erupted third molars. Aspects of the study design, such as selection of participants, might have had an impact on differing findings. The strengths of the SHIP are the population-based design, the high level of quality assurance, the use of MRI, and strict adherence to standardization of examination methods and data management.[23] Two trained dentists, who strictly adhered to the standardization, performed the MRI protocol developed for analyzing third molars. Taken together, high-quality data standards were consistently assured. Limitations to the study also must be considered. According to quality guidelines of the federal committee on MRI (Gemeinsamer Bundesausschuss), a slice thickness of 5 mm is recommended for head and neck diagnostics.[30] This study used a 4-mm slice thickness, and the gap between 2 slices was 10%. Therefore, it was not possible to differentiate between soft tissue and bony impaction of third molars.[11] This also could have led to misclassification between partially erupted and impacted third molars, which could have influenced the present results. To detect third molars in a whole-body MRI, the use of a 3-dimensional MR dataset with dental reconstructions perpendicular to the mandible and maxilla might have increased diagnostic yield. However, the observer agreement for third molar assessment was very good.

In contrast to Graziani et al[2] who described an association of impacted third molars with triglycerides, HDL cholesterol, and glucose, the authors

could not confirm such associations; similarly, the authors did not find an association of impacted third molars with increased levels of cholesterol and HDL cholesterol. Unexpectedly, the authors detected an inverse association of erupted third molars with leptin. Leptin is secreted by adipocytes, acts in the hypothalamus to suppress appetite and food intake, and regulates body weight.[17,31] Serum leptin concentrations have been positively related to obesity and metabolic syndrome.[32] Leptin is a mediator of the inflammatory response, produces proinflammatory cytokines in obesity,[33] and has a proinflammatory effect in the immune system.[20] In the present study, participants with erupted third molars had the lowest concentrations of leptin. This inverse association could be connected to the crucial role of teeth development for digestion in evolution. The masticatory benefits of food processing and carnivorous selection could be reasons for the evolution of smaller teeth and jaws.[34] Consistent with these findings, Um et al[35] postulated that leptin could act as an important modulator of dental mesenchymal stem cells and would have a promoting effect on cemento-blastic and odontoblastic differentiation. Impaction or eruption of third molars could be linked with leptin serum levels in a kind of negative feedback mechanism. The authors also found that participants with third molars had lower serum levels of Ang-2 than participants without third molars. The angiopoietin and TIE system and the release of Ang-2 have relevance in systemic inflammatory disorders by regulating inflammation.[18] However, the authors cannot explain the inverse associations of third molars with the angiopoietin and TIE system.

The results did not substantiate a relation between third molars and an increase in systemic inflammatory markers. Therefore, dental practitioners should be careful when considering this as the only indication for third molar removal, especially in medically compromised patients. The authors found that participants with erupted third molars had lower levels of messenger peptide hormones, such as leptin and Ang-2, compared with patients without third molars.

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